Journal of Medical Virology 59:541–546 (1999)

Estimating the Time of HTLV-I Infection Following Mother-to-Child Transmission in a Breast-Feeding Population in Jamaica

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Mother-to-child transmission of human T-cell lymphotropic virus type I (HTLV-I) is primarily due to prolonged breast-feeding (>6 months) in the postnatal period. Most infant infections are not identifiable until 12 to 18 months of age by available whole virus Western blot serologic tests because of their inability to distinguish passively transferred maternal antibody from infant antibody. We investigated two methods to assess more accurately the time of infant infection. In prospectively collected serial biospecimens, HTLV-I–specific immunoglobulin (Ig) isotypes of IgM and IgA were determined by Western blot and HTLV-I proviral DNA was detected by polymerase chain reaction (PCR). IgA and IgG reactivity was assessed in periodic serum samples from 16 HTLV-I–seropositive children while IgM reactivity was assessed in 9 of the 16 children. Approximately three to five samples were tested for each child. IgG reactivity was observed in 100% of children at 24 months of age and 73% of children at 6–12 months of age; however, this could represent maternal and not infant antibody. Both IgA and IgM reactivity were insensitive indicators of infection, with only 50% of children showing reactivity at 24 months of age. PCR testing was performed in biospecimens obtained from 11 of these children. An estimated median time of infection of 11.9 months was determined by PCR, which was similar to the median time to infection determined by whole virus Western blot (12.4 months; $P = 0.72$). PCR tests support a median time to infection that is similar to that estimated by whole virus Western blot. ***J. Med. Virol. 59:541–546, 1999.***

KEY WORDS: HTLV-I; vertical transmission; infants; seroconversion; immunoglobulins; polymerase chain reaction

INTRODUCTION

Early life infection with HTLV-I has been implicated as the most important risk for subsequent development of the aggressive, non-Hodgkin's lymphoma, adult T-cell leukemia/lymphoma (ATL). Approximately 50% of non-Hodgkin's lymphoma cases could be prevented by elimination of childhood HTLV-I infections in endemic areas [Manns et al., 1993]. The majority of childhood infections are thought to occur by mother-to-child transmission in the postnatal period via breast-feeding, with confirmed transmission rates in the 18%–39% range [Tsuji et al., 1990; Wiktor et al., 1997]. The risk of transmission increases with longer duration of breast-feeding (> 6 months), while shorter duration appears to correlate with a decrease in risk [Hino et al., 1995; Wiktor et al., 1997]. However, most infant infections are not identifiable until 12 to 18 months of age, due to the inability of available standard whole virus serologic tests to distinguish passively transferred maternal anti–HTLV-I IgG from infant antibodies. Maternal antibody appears to be protective in the early period following birth [Takahashi et al., 1991], but not sufficient to protect against infant infection in the setting of prolonged breast-feeding.

Earlier diagnosis of infant seroconversion and its relationship to breast-feeding duration and other risk factors associated with maternal transmission would be useful information, particularly for HTLV-I endemic

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Accepted 5 May 1999

developing countries. In these countries, the implementation of breast-feeding intervention programs or use of artificial feedings to prevent mother-to-child transmission may have adverse effects on infant nutritional status, infectious disease morbidity, and mortality. Accurately determining the time of HTLV-I infection in children of HTLV-I–seropositive mothers would assist in our understanding of the underlying mechanisms of infection, in the assessment of risk factors, and ultimately in the benefits of certain interventions for primary prevention of infection and disease.

HTLV-I infection in children (via mother-to-child transmission) has usually been determined by a combination of enzyme-linked immunosorbent assay (ELISA) and confirmation with whole virus Western blot that measures predominately IgG antibodies. In prior studies [Takahashi et al., 1991; Wiktor et al., 1997], the infection time was estimated as the midpoint between the time of weakest (or last negative) Western blot and the time of the next stronger Western blot. In actuality, this may underestimate the time of earliest infection in the infant, since the Western blot results are based on competing levels of antibodies in infected children—IgG from the mother that crosses the placenta and the child's own innate antibody production.

In the current investigation, we evaluated two methods to determine more accurately the time of infection in infants. First, we evaluated sera for HTLV-I viral-specific immunoglobulins with the thought that the humoral immune response to IgM and IgA would be specific for infant infection rather than maternal, and then, by polymerase chain reaction, we detected proviral DNA as a more precise estimate of time of infant infection.

MATERIALS AND METHODS

Study Population

This study is a further examination of HTLV-I–seropositive children from a study of mother-to-infant transmission conducted in Jamaica in 1989–1990 [Wiktor et al., 1997]. Enrollment in the original study included 212 seropositive women and 145 seronegative women attending two large public antenatal clinics in Kingston, Jamaica, in order to evaluate the risk of HTLV-I infection in early life. Following delivery, mothers and their children were seen at a research clinic every 6 weeks for the first 6 months and thereafter every 3 months. During the course of the study, 28 children born to seropositive women (and no children born to seronegative women) were persistently HTLV-I–positive, having been infected either at birth or during the period of breast-feeding. The study was approved by the Human Subjects Review Boards of the University of the West Indies and the U.S. National Cancer Institute.

We were able to analyze HTLV-I–specific immunoglobulin (Ig) isotypic antibodies for 16 of the 28 seropositive children by Western blot assay. Due to the limited volume of infant serum samples, HTLV-I–specific Ig isotype assays were not performed for 12 of the 28 children. However, it was known that these 12 children had a median breast-feeding time of 25.9 months (interquartile range, IQR, of 17.0–30.5), almost twice the median breast-feeding time of the 16 children examined here, 13.75 months (IQR of 9.1–18.0). These 16 children with shorter breast-feeding times provided an opportunity to identify the earliest time of infection in children with limited exposure.

Periodic serum samples obtained from each of the 16 children were tested with the first sample between the ages of 4.8 months and 12.6 months (median of 5.9 months, IQR of 5.7–6.1) and the last available sample between the ages of 21.7 months and 36.5 months (median of 27.45 months, IQR of 23.5–30.3 months). Approximately three to five samples (median of four) for each child were tested. Within the group of 16 children tested for Western blot Ig antibody reactivity, all 16 were tested for anti–HTLV-I IgA and IgG while, due to limited volume of samples, only 9 were tested for anti–HTLV-I IgM. We also analyzed serum samples from a seronegative infant born to a seropositive mother for HTLV-I–specific IgG, IgA, and IgM. These control samples were obtained at the ages of 3.2, 6.1, 11.2, and 13.9 months.

Laboratory Methods

Seroconversion was established by first testing serum samples with HTLV-I enzyme-linked imunosorbent assay (ELISA) (Cambridge Bioscience, Cambridge MA; Dupont, Wilmington DE; or Genetic Systems, Seattle WA). Samples found to be positive were confirmed by whole virus HTLV-I Western blot enhanced with a recombinant envelope protein (r21e) (Cambridge Bioscience). Samples were considered positive if *gag* (p19 and/or p24) bands and envelope (*env*) bands (gp46 and/or r21e) were detected. The precise methodology for estimating time of seroconversion was described previously [Wiktor et al., 1997]. Serial serum samples were tested and the estimated time of HTLV-I infection was computed by calculating the number of months from birth to the midpoint between the child's last negative Western blot and the first positive. Maternal antibody titer was measured on the enrollment sample by the endpoint dilution method using ELISA testing with threefold dilutions.

Serum samples were tested for HTLV-I–specific isotypic antibodies based on a method described previously [Lal et al., 1993]. Briefly, r21e-spiked Western blot strips (Cambridge-Biotech, Rockville, MD) were sequentially probed under identical conditions, first with a child's serum, then with a mouse monoclonal antibody (mAb) specific for human IgG (clone HP6017), IgA (clone HP6123), or IgM (clone HP6083) (Hybridoma Reagent Laboratory, Kingsville, MD), and finally by incubation with alkaline phosphatase-conjugated antimouse IgG as the third antibody. The Western blot assay tested samples for antibody reactivity to *gag* (p19, p24) and *env* (r21e) proteins in three HTLV-I–specific Ig isotypes (IgG, IgA, and IgM). Each Western

blot plate was run with four to five infant samples and one sample from a negative adult control.

Polymerase chain reaction (PCR) testing was performed on lymphocyte samples for 11 of the 16 HTLV-I–seropositive children with at least one negative Western blot prior to seroconversion. DNA from samples was amplified using HTLV-I biotinylated tax primers SK-43 and SK-44. This PCR method can detect 10–30 proviral DNA copies per 100,000 lymphocytes. We tested two samples per subject. Lymphocyte samples were selected based on each individual child's Western blot profile taken in terms of the narrowest window for the estimated time of seroconversion. For eight of these children, a PCR-negative sample was obtained as well as a PCR-positive sample, and the PCR-based time of infection for each child was defined as the midpoint between the respective times these lymphocyte samples were obtained. For three other children, the samples available for testing yielded only PCR-positive results and so for these three only the first PCR-positive sample was relevant. The PCR-based time of infection for these latter three is defined as the midpoint between birth and the time the first PCR-positive lymphocyte sample was obtained.

Statistical Methods

Frequency computations of reactivity of individual viral proteins were performed with Lotus 1-2-3 (Lotus Development, Cambridge, MA). The Spearman rank-order statistic was used to examine correlations between age at which breast-feeding ceased, maternal antibody titer, and estimated time of infection. The Wilcoxon sign rank test was used to test for differences in the estimated time to HTLV-I infection between the PCR and Western blot methods. Statistical computations were performed with mainframe SAS Version 6.08 (Statistical Analysis System Institute, Cary, NC).

RESULTS

The results of anti–HTLV-I–specific isotype assay were separated (based on the time the serum sample was obtained) into four 6-month intervals from birth up to 2 years, plus an additional interval for the results of samples obtained at the 24-month point and later (up to 36.5 months). In Figure 1, the percent reactivity in each interval for IgG, IgA, and IgM are shown.

IgG reactivity was first evident in the first 6 months of life, yet only 40% of samples obtained during this period were positive. There was no difference in reactivity among the three HTLV-I antigens p24, p19, and r21e. Between 6 to 12 months of age, IgG reactivity was much more prominent, though only 60% of serum samples reacted to p24, while 73% reacted to both p19 and r21e. Samples obtained between ages 12 and 18 months showed a prevalence of IgG reactivity to all three antigens similar to the previous period with more samples positive for p19 (71%) and r21e (79%) than p24 (57%). The fourth time period, 18 to 24 months of age, showed reactivity to p24 (80%), p19, and r21e (both 87%) that was greater than at earlier ages. As would be expected in seroconverting children, all eventually showed reactivity to IgG after 24 months of age.

There was no IgA reactivity in the first 6 months, but reactivity to each antigen increased over time, although with a lower response than IgG. There was low reactivity to all three antigens between 6 to 12 months of age (6% for p24 and r21e, 13% for p19) and 12 to 18 months of age (reactivity to all three is 20%). Reactivity substantially increased in samples obtained from 18 to 24 months of age, yet still only 47% showed reactivity to p19, and even fewer samples reacted to p24 (27%) and r21e (20%). Although after 24 months of age, higher IgA reactivity of samples to each antigen were observed, no more than 50% of children's serum samples showed reactivity at this point.

The pattern of IgM reactivity was similar to that of IgA. IgM reactivity to the three antigens, which was nonexistent in samples taken before 6 months of age, appeared between 6 and 12 months of age but only for p19 (40% of samples) and p24 (10% of samples). Reactivity to r21e first appeared in samples taken between 12 and 18 months of age and had the same response as p24 (29%), while reactivity to p19 was increased at 43%. Samples taken from 18 to 24 months of age showed no increase in reactivity to p24 and p19, and reactivity to r21e disappeared completely. Samples obtained after 24 months of age showed a renewed increase in IgM reactivity to all three antigens, with reactivity to p24 and p19 peaking at 50% of samples tested while one-third of samples showed reactivity to r21e.

Control samples obtained at 6.1, 11.2, 23.1, and 29.1 months of age, from a seronegative infant born to a seropositive mother, all tested negative for HTLV-I–specific IgG, IgA, and IgM antibodies to p19, p24, and r21e. Samples obtained from this infant at 3.2, 6.1, 11.2, and 13.9 months of age were also tested with the whole virus Western blot. Samples at 3.2 and 6.1 months were positive, while samples from 11.2 and 13.9 months were negative, indicating the possible disappearance of maternal antibodies.

Generally, in children born to a seropositive mother, the whole virus Western blot and the HTLV-I–specific IgG Western blot were in agreement except in the first 6 months. The whole virus Western blot was more likely to be seropositive during this period compared to the HTLV-I–specific Western blot (data not shown). There were no seropositive children born to a seronegative mother in this study when evaluated by the whole virus Western blot.

We subsequently estimated the time of infection using PCR detection to compare this method to the results of HTLV-I–specific Ig isotypes by Western blot or whole virus Western blot. A direct comparison of the two methods is presented in Table I. In 4/11 (36%) children evaluated, the estimated time of infection was detected earlier by PCR than by whole virus Western blot. The median estimated time to infection for PCR was 8.7 months (IQR of 7.3–13.5), while the median time to seroconversion by whole virus Western blot was

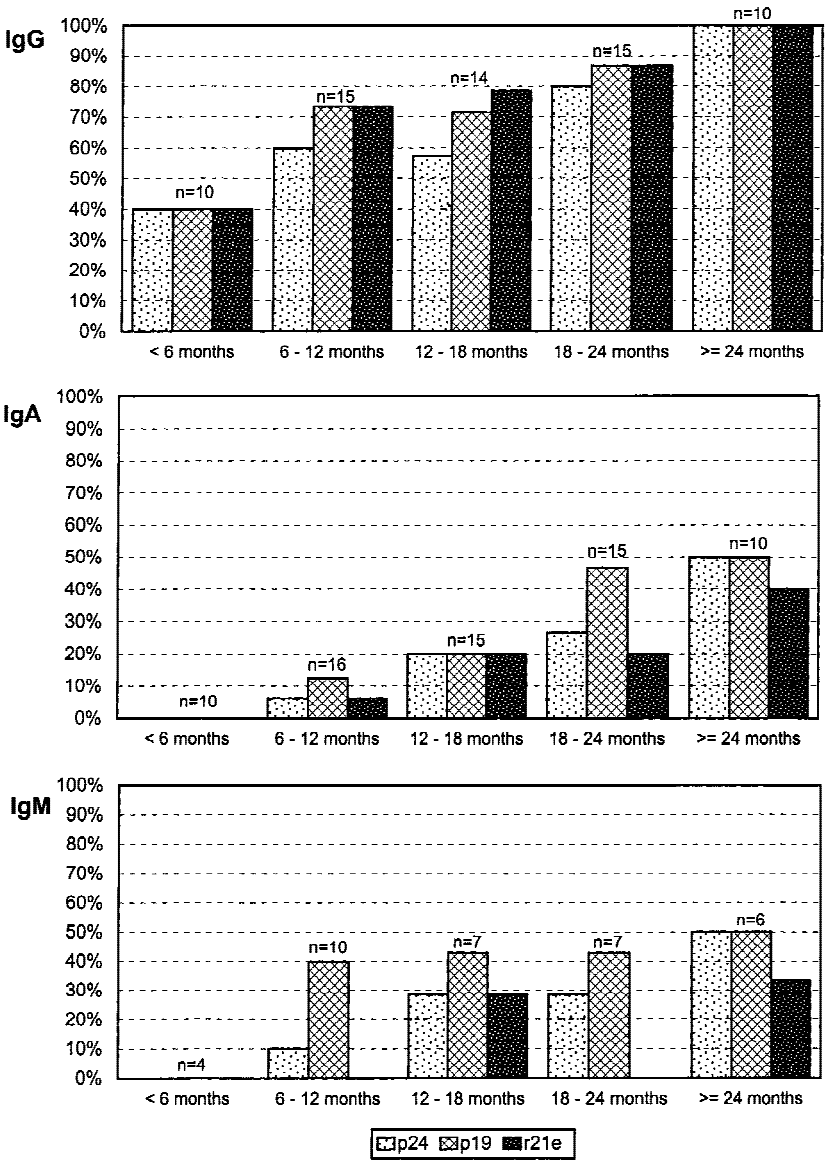

Fig. 1. HTLV-I–specific Ig in serum samples. Percent reactivity in each period to *gag* (p24, p19) and *env* (r21e) antigen was calculated for IgG, IgA, and IgM. Percent reactivity is defined as number of samples showing reactivity to an antigen divided by total samples tested (shown above each time period) for the particular HTLV-I–specific Ig antigen during that time period.

10.3 months (IQR of 9.3–14.4). However, when we restricted analysis to children whose time of infection was based on the midpoint between a PCR-negative and subsequent PCR-positive sample (n = 8), there was no significant difference in the median time of infection between these two methods [11.9 months (IQR of 8.7–15.8) vs. 12.4 months (IQR of 8.6–16.4; P = 0.75)]. For the majority of cases, we found a strong concordance between PCR and whole virus Western blot results, with very little Western blot reactivity in the PCR-negative samples tested. The Western blot reactivity observed in the PCR-negative samples most likely represented maternal IgG that was placentally transferred. For comparison with time of infection, we included data on age breast-feeding stopped and maternal titer at delivery in Table 1.

DISCUSSION

Understanding the characteristics of mother-to-child transmission of HTLV-I infection is important to our discovery of the mechanisms crucial in the development of HTLV-I–associated diseases. Risk factors for mother-to-child transmission include long-term breast-feeding (> 6 months), high maternal antibody titer, long duration (> 4 hr) of membrane rupture, and lower maternal income [Wiktor et al., 1997]. This study examined the early humoral immune response of 16 HTLV-I–positive children to HTLV-I–specific immunoglobulin isotypes following birth. In addition, polymerase chain reaction was applied to determine more precisely the time of infection.

Among the children tested, anti–HTLV-I IgG had the

TABLE I. Estimated Times of HTLV-I Infection and Seroconversion, Age at Last Breast-Feeding and Maternal HTLV-I Titer[a]

Subject	Estimated time of infection based on PCR results (months)	Estimated seroconversion time based on standard Western blot whole virus assay (months)	Age breast-feeding stopped (months)	Maternal titer at delivery
A	5.7	12.9	3.8	4,067
B	5.8	9.8	7.1	5,279
C	6.3	9.3	9.1	4,233
D	8.1	8	7.9	6,874
E	8.7	8.6	14.1	27,513
F	10.3	10.3	10.2	6,991
G	7.3	7.3	18	4,057
H	13.5	16.4	10.3	43,740
I	15.8	14.4	17	43,740
J	21.4	21.4	42	13,905
K	21.5	21.5	13.4	20

[a]For subjects A, B, and C, the first PCR sample was positive; estimated time to infection is the midpoint from birth to time of first sample.

strongest response. However, it was least reliable from a diagnostic standpoint in the newborn, since maternal IgG passes through the placenta during gestation and we were unable to distinguish maternal IgG from that of the infant. Our results indicated that anti–HTLV-I IgA reactivity, a better marker of early infection in the infants, increased gradually as a percentage of positive samples but had low sensitivity overall showing reactivity in only 50% of samples after 24 months of age. Anti–HTLV-I IgM reactivity was higher than IgA early on, yet like IgA, the percent reactivity remained far less than that observed for IgG. The results in this study of infants sharply contrast with that of the immune response to HTLV-I Ig isotypes in an adult population [Manns et al., 1994]. Using the same assay in adults, we demonstrated strong antibody responses to HTLV-I–specific IgG, IgA, and IgM. In the adult study, 60% to 80% of samples showed IgG, IgA, or IgM reactivity to presumably viral proteins in the first 6 months following HTLV-I exposure by transfusion. These data suggest that the assay lacks sensitivity in infants. This may be due to diminished antibody response or low-level antibody production in infants [Ichijo, 1995]. Alternatively, it could be due to a limitation of our assay method, since unlike some HIV studies, we did not use the technique of IgG depletion that appears to enhance sensitivity for detecting anti-HIV IgA and IgM in infants [Schupbach et al., 1989].

Despite the low sensitivity of IgA and IgM, the patterns of reactivity were instructive. There was consistency of results over consecutive serial samples assayed. All subjects with initial samples that showed IgA reactivity had persistent positivity in subsequent samples. On the other hand, there was some evidence of variability among IgM reactivity. The early samples of three children showed IgM reactivity to some HTLV-I antigens while later samples did not show reactivity to the same HTLV-I antigens. Thus, we observed that IgM reactivity was sometimes transient. It also should be noted that the median length of time that IgM (directed against any HTLV-I antigen) persisted in the six IgM-positive children in our study was 12.5 months, with five of the six subjects' final serum samples still showing IgM reactivity to at least one HTLV-I antigen. The continued presence of detectable levels of anti–HTLV-I IgM over relatively long periods of time was not due to new viral exposure from breast-feeding since the majority of IgM-positive children were weaned either before or shortly after their first IgM-positive sample. As previously reported, the persistent IgM was probably due to continued HTLV-I viral replication [Manns et al., 1994].

A number of studies have documented the immune responses of infants born to human immunodeficiency virus type-1 (HIV-1) infected mothers [Weiblen et al., 1990; Landesman et al., 1991; Martin et al., 1991; Quinn et al., 1991; Kline et al., 1994; Schupbach et al., 1994]. Some studies indicated that the presence of anti–HIV-1 IgA antibody was a sensitive and also a highly specific method of assessing HIV-1 status in children of HIV-1 positive mothers [Weiblen et al., 1990; Landesman et al., 1991; Martin et al., 1991; Quinn et al., 1991; Schupbach et al., 1994]. In one HIV-1 study [Weiblen et al., 1990], 64 serum samples from infants, 2 less than our study, were tested for anti–HIV-1 IgA antibodies and the earliest samples showing reactivity were obtained from infants less than 3 months of age. In that same study, the 64 samples also were tested for anti-HIV IgM antibodies and the earliest samples showing reactivity again were from infants less than 3 months of age. The differences observed may be related to the fact that HIV-1 infection almost always occurs in the peripartum period (in utero or intrapartum) [John and Kreiss, 1994], whereas HTLV-I infection occurs predominantly in the postnatal period associated with prolonged breast-feeding [Wiktor et al., 1997]. As previously mentioned, the assay method employed in the HIV studies differed from ours since IgG depletion was performed prior to assaying for anti-HIV IgA and IgM.

Since IgA and IgM did not prove to be a sensitive early marker of HTLV-I infection, we evaluated time of infection using PCR to detect proviral DNA in 11 of the 16 infants. We found a strong agreement between the PCR and whole virus Western blot results. There was infrequent whole virus Western blot reactivity in the

PCR-negative samples and this reactivity was confined to IgG that was probably placentally transferred. After determining the time of infection with PCR, we were able to examine the subjects' estimated time of infection with data on duration of breast-feeding and maternal HTLV-I antibody titer. These data suggested that most infant infections occurred with prolonged breast-feeding. However, there were some infant infections associated with short breast-feeding times (< 8 months). Some infections also occurred when maternal antibodies were presumably protective. These infections may have occurred in utero or intrapartum.

Current recommendations for prevention of maternal transmission of HTLV-I in Japan, the U.S., and other developed countries are to provide artificial feedings for infants of HTLV-I–seropositive mothers [Centers for Disease Control and Prevention, 1993; Hino et al., 1996]. Other data suggest that breast-feeding cessation at the age of 6 months or earlier may also reduce the risk of infection [Takezaki et al., 1997]. This alternative may be a more feasible approach for some developing countries due to concerns of infant nutritional status and the associated increase in morbidity and mortality in the absence of breast-feeding. Thus, in this setting, limited breast-feeding should be encouraged. Unfortunately, none of the currently available interventions can eliminate infections 100% of the time, since even bottle-feeding results in infant infections of about 3% [Hino et al., 1996]. Therefore, we should continue to explore alternative approaches for the primary prevention of HTLV-I infection associated with vertical transmission.

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